

ABSTRACT

EXTERNAL CONTROL REAGENTS
FOR NUCLEIC ACID AMPLIFICATION

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External control reagents for nucleic acid amplification are provided that verify the absence or presence of specific target sequences, and correct primers and probes. A single-stranded, external control polynucleotide is amplified with primers of the same sequence as target primers. Probes with detectable labels and sequences specific for target and external control polynucleotides allow for detection and measurement. The primers and the detectable probe are adjacent or substantially adjacent when hybridized to the external control polynucleotide. Target and control amplicons may be detected by increased fluorescence induced by polymerase-mediated 5' nuclease cleavage or hybridization of a self-quenching probe complementary to both target and external control polynucleotides. A kit of PCR reagents can be dispensed into vessels for rapid and accurate nucleic acid amplification assay, with real-time or end-point measurements. The amplification control reagents, kits, and methods of the present invention provide positive and negative control tests which can be conducted concurrently with target amplification. Allelic differences at genetic loci can be detected, including single nucleotide polymorphisms (SNP).

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